OPTIMIZATION OF GLUCOSE PRODUCTION FROM SUGARCANE BAGASSE USING RESPONSE SURFACE METHODOLOGY (RSM)

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ABSTRACT

Glucose benefits much for industry, medical field and researches. Old method in producing glucose involved chemical process throughout the procedures. This method is not environmental friendly. Research showed that the production of glucose was successfully obtained from plant biomass. The purpose of this study was to optimize the glucose production from sugarcane bagasse using Response Surface Methodology (RSM). Bagasse is the sugarcane residue after juice extraction. The parameters used in this research were temperature, substrate (cellulose from bagasse) dose and enzyme (cellulase from *Aspergillus niger*) dose. The bagasse was treated with alkali before enzymatic hydrolysis procedure took place for glucose production. Screening process conducted in this study was to determine the best range of parameters involved and this range would be used for the optimization using RSM. Seventeen experiments have been arranged by RSM for analysis. RSM predicted the best conditions of parameters were 45°C of temperature, 1.3 g of substrate dose and 0.8 g of enzyme dose with the glucose production was 5.8672 g/L. The validation of experiment showed that glucose production was 5.725 g/L compared to predicted value, 5.8672 g/L. Before optimization, the production of glucose was 1.010 g/L with conditions 45°C of temperature, 2.0 g of substrate dose and 1.0 g of enzyme dose. The percentage of increment was 82.36%. From these observation and analysis, it can be concluded that the objective of this research to optimize the glucose production from sugarcane bagasse using Response Surface Methodology (RSM) was successfully conducted.

ABSTRAK

Glukosa mempunyai banyak kegunaan dalam industri, bidang perubatan and pengkaji. Kaedah lama dalam penghasilan glukosa dari bahan mentah sehingga terhasilnya glukosa keseluruhannya melibatkan proses tindak balas kimia. Kaedah ini tidak mengutamakan alam sekitar. Kajian terdahulu menunjukkan penghasilan glukosa dari serat tumbuhan telah berjaya dilakukan. Kajian ini bertujuan untuk mengoptimumkan penghasilan glukosa daripada hampas tebu menggunakan Kaedah Tindak Balas Permukaan (RSM). Hampas tebu adalah sisa-sisa daripada tebu selepas penghasilan air tebu. Pembolehubah-pembolehubah yang digunakan dalam kajian ini adalah suhu, dos substrat (selulosa daripada hampas tebu) dan dos enzim (enzim selulase daripada *Aspergillus niger*). Hampas tebu tersebut telah dirawat menggunakan alkali sebelum melalui proses hidrolisis menggunakan enzim dalam penghasilan glukosa. Proses saringan yang dijalankan dalam kajian ini bertujuan untuk mendapatkan lingkungan parameter terbaik untuk dioptimumkan di dalam RSM. Sebanyak 17 eksperimen telah disusun oleh RSM untuk dianalisis. RSM meramalkan pembolehubah terbaik untuk penghasilan glukosa adalah pada suhu 45°C, 1.3 g dos substrat dan 0.8 g dos enzim dengan penghasilan glukosa adalah 5.8672 g/L. Eksperimen telah dijalankan dan didapati penghasilan glukosa adalah sebanyak 5.725 g/L berbanding yang diramalkan iaitu sebanyak 5.8672 g/L. Sebelum proses ini dioptimumkan, penghasilan glukosa hanyalah sebanyak 1.010 g/L dengan suhu 45°C, 2.0 g dos substrat and 1.0 g dos enzim. Peratus kenaikan penghasilan glukosa adalah sebanyak 82.36%. Berdasarkan pemerhatian dan analisis, dapat disimpulkan bahawa objektif kajian ini untuk mengoptimumkan penghasilan glukosa daripada hampas tebu menggunakan RSM telah berjaya dilakukan.

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LIST OF SYMBOLS/ABBREVIATIONS

ANOVA	-	Analysis of Variance
CCD	-	Central Composite Design
CCRD	-	Central Composite Rotable Design
DNS	-	Dinitrosalicylic
g	-	gram
g/L	-	Gram per Litre
L	-	Litre
mg	-	miligram
mL	-	mililitre
RSM	-	Response Surface Methodology
°C	-	Degree Celcius
µL	-	microlitre
%	-	Percentage

CHAPTER 1

INTRODUCTION

1.1 Introduction

Sugarcane or its scientific name *Saccaharum officinarum* is one type of the lignocellulosic biomass which is abundant and can be easily found. The solid residue left after extraction of the juice is called sugarcane bagasse or generally called as bagasse. It has high potential or the production of bioethanol, biohydrogen or other biofuel. The use of sugarcane bagasse in chemistry and biotechnology has been recently reviewed (Pandey *et al*., 2000).

Generally, in the bagasse specifically or other biomass's cell wall, it contains lignin, hemicelluloses and celluloses. Lignin can be hydrolyzed into poly aromatic hydrophobic structure for renewable solid fuel, while hemicelluloses can be hydrolyzed into five carbon (C_5) sugars for bio-refineries or enzymes production. In this research, the desired component is the cellulose which can be hydrolysed into six carbon (C_6) sugars which is glucose for bioethanol or other renewable liquid fuel production.

Generally, there are two main procedures in order to produce glucose from sugarcane bagasse. The first one is the pretreatment of the bagasse and the second one is the enzymatic hydrolysis itself. Many pretreatment methods have been reported and several detailed review papers have been published (Sun and Cheng, 2002). The pretreatment process indicates positive impact on the cellulose hydrolysis and consequently the glucose yields. The purpose of the pretreatment is to separate lignin and hemicelluloses from cellulose, reduce cellulose crystallinity and increase the porosity of the lignocellulosic materials so that cellulose hydrolysis can be significantly improved (Kuo and Lee, 2009).

Hydrolyzing the cellulose into glucose is performed by using specific enzyme in certain conditions such as temperature, the concentration of enzyme, the quantity of cellulose, pH and time. These conditions could be optimized using Response Surface Methodology (RSM) in order to get the optimum data. The enzymatic hydrolysis had been done on waste paper office using three commercial cellulases, Acremonium cellulase, Meicelase and Cellulosin T2 (Park *et al*., 2002). Waste paper office contains cellulose. This is one of the methods to reduce the waste paper office and consequently turn it into energy sources.

The optimization in RSM has demonstrated the use of a central composite factorial design by determining the optimum conditions leading to the high yield of enzyme production. Thus, smaller and less time consuming experimental designs could generally suffice for the optimization of many fermentation processes (Adinarayana and Ellaiah, 2002). With the aid of the experimental design and response surface methodology, the optimal concentrations of sugarcane molasses, bacteriological peptone and yeast extract Prodex Lac SD® for the production of glucosyltransferase by *Erwinia* sp. D12 were found to be 160 g/L, 20 g/L and 15 g/L, respectively (Kawaguti *et al*., 2006).

1.2 Problem Statement

The bagasse is the major residue from the sugar and alcohol industry. It can be a source of pollutant if it is dumped before treatment. The bagasse has no value to those industries because it is considered as a waste. As an engineer, everything has its own value including the wastes and a research is needed in order to develop the potential. A lot of studies have been done on treating the bagasse into something valuable.

The depleting fossil fuel resources and the environmental problems with greenhouse gases have sprung forth the awareness of the importance of renewable and cleaner sources of energy, such as biogas produced from lignocellulosic biomass. Sugarcane bagasse is a cheap and abundant raw material which can be used for this purpose (Carvalho, 2009).

From previous studies, the bagasse is found out to be a fuel source as it can produce more heat energy to supply in sugar and alcohol industries themselves (Sendelius, 2005). Besides, the researchers also found out that the bagasse can also produce glucose as it contains a lot of cellulose in its fibre. The major challenge in this research is to convert economically the bagasse into glucose. This research will focus on enzymatic hydrolysis process with various parameters to find out the most economic and optimum conversion.

1.3 Objective

The research was proposed to optimize the production of glucose from sugarcane bagasse using Response Surface Methodology (RSM).

1.4 Scopes of Study

In order to achieve the stated objective, the following scopes of study have been identified:

a) To study the effect of temperature on production of glucose.
b) To study the effect of cellulose dose (substrate) on production of glucose.
c) To study the effect of cellulase dose (enzyme) on production of glucose.
d) To optimize the glucose production using Response Surface Methodology (RSM).

1.5 Rationale and Significance

As mentioned previously, almost the entire quantity of the bagasse produced is used by the sugar mills themselves as fuels or boilers, which is a necessity-based on economical and efficient application. However, process such as production of enzymes and other products utilizing the bagasse as solid substrate would need relatively a small fraction of total bagasse. This may not affect its supply to the sugar mills and thus appears attractive for bioprocess (Pandey *et al.*, 2000).

About 32% of bagasse is produced from every tone of sugarcane that has been produced. The total plantation area of sugarcane bagasse in Malaysia is nearly 34 500 acre. About 1 111 500 tonnes of sugarcane is produced in 2002, hence the bagasse can be easily obtained in Malaysia (Lee and Mariatti, 2008). This research could be done without any problem in looking for raw materials.

This study is to optimize the production of glucose from sugarcane bagasse using Response Surface Methodology. This optimization is to find the best parameters in converting the bagasse into glucose economically. This leads to achieve the 'waste into wealth' industrial concept nowadays. More money can be generated from the waste. This research comply this recent needs.

The importance of this research is the glucose as the raw material of ethanol production or other biofuels. The other significance of the study is to reduce the environmental pollution as it is a worldwide threat to public health has given rise to a new massive industry for environmental restoration.

CHAPTER 2

LITERATURE REVIEW

2.1 Sugarcane Bagasse

Sugarcane is one of the agricultural products that have many benefits to human usage and also other living things (Pandey *et al*., 2000). Sugarcane is used as a main source in producing sugar for food and beverages. In sugar and alcohol industry, sugarcane bagasse is generated as a waste. This bagasse could be a pollutant to the environment if it is just disposed without treatment. Generally, the biomass composed of cellulose, polyoses, lignin, hemicelluloses, small amounts of extractives and mineral substances. In the cell wall of biomass, it consists of lignin, hemicelluloses and cellulose. The sugars of which they are made are linked together in long chains called polysaccharides, which form the structural portion of plant cell walls.

Unraveling these complex polymeric structures is the key to economic biorefining. Cellulose microfibrils consist of a crystalline structure of thousands of strands, each of which contains hundreds of glucose sugar molecules. These microfibrils are wrapped in a sheath of hemicelluloses and lignin, which protects the cellulose from

microbial attack. Hemicelluloses are relatively easy to break down using pretreatment step. It also disrupts the hemicelluloses or lignin sheath around the cellulose, making the cellulose accessible to further hydrolysis. The hydrolysis of the lignocellulosic biomass will liberate C_6 fermentable sugars will be carried out via enzymatic hydrolysis (Camassola and Dillon, 2009). Figure 2.1 below shows the structure of cellulose microfibrils.

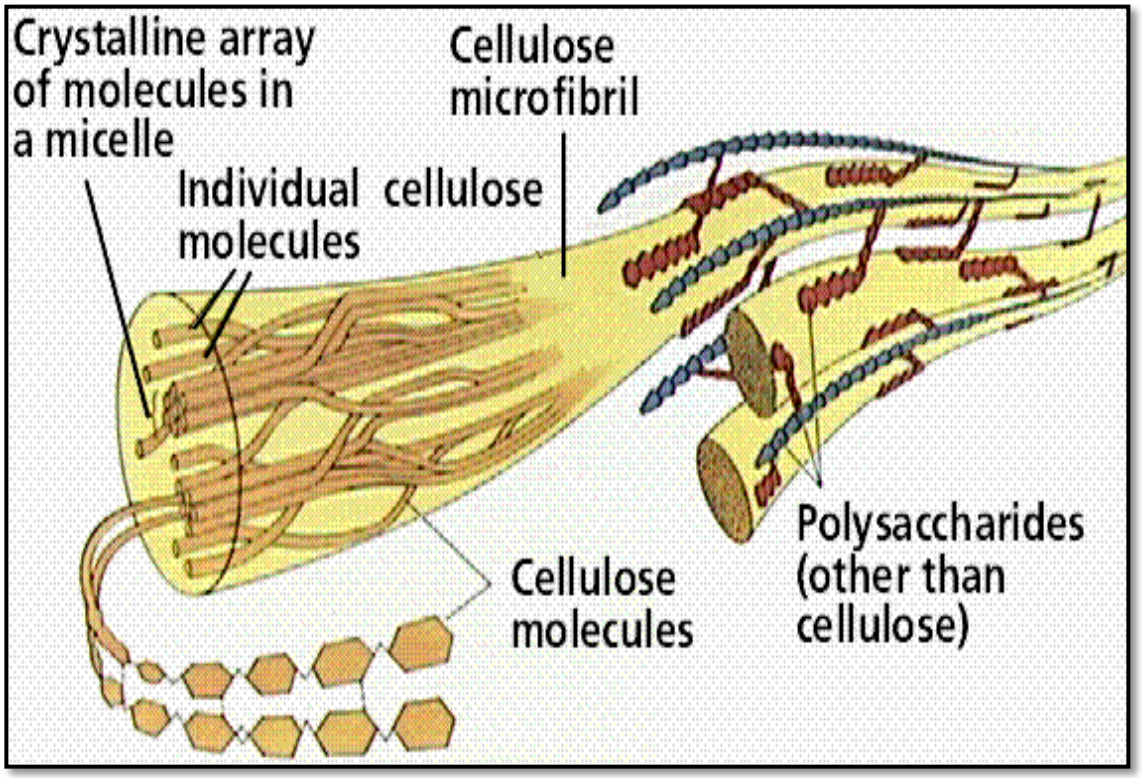

Figure 2.1: The structure of cellulose microfibrils

In this study, the bagasse is chosen as it consists of approximately 50% cellulose and 25% each of hemicelluloses and lignin. Chemically, bagasse contains about 50% α-cellulose, 20% pentosans and 2.4% ash. Because of its low ash content, bagasse offers numerous advantages in comparison to other crop residues such as rice straw and wheat straw, which have 17.5% and 11% respectively (Pandey *et al.*, 2000). Low ash content will enhance the enzymatic hydrolysis process. Sugarcane bagasse ash, a byproduct of

sugar and alcohol production, is a potential pozzolanic material which is used as partial replacement of Portland cements in mortars and concrete (Cordeiro *et al.*, 2009). Figure 2.2 shows the structure of cellulose in the plant cell wall.

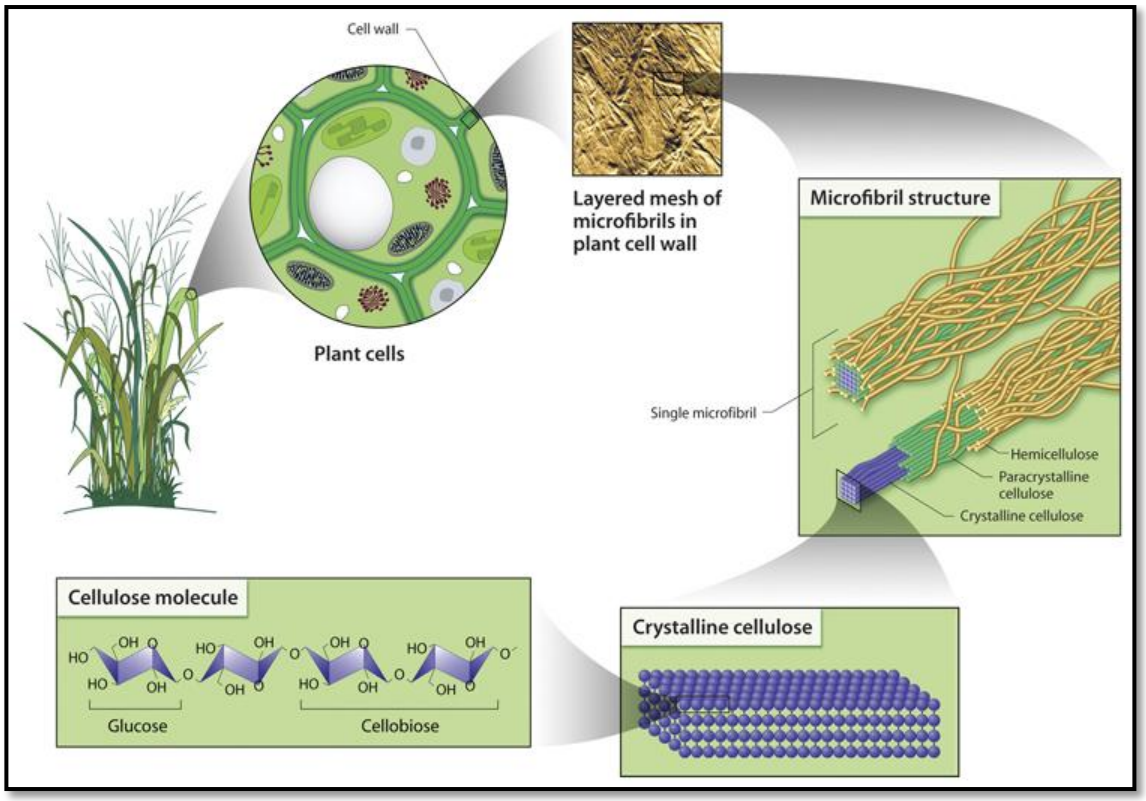

Figure 2.2: Zooming into the views inside the plant cells; structure of cellulose molecule

2.2 Pretreatment Method

Before the enzymatic hydrolysis is performed, the bagasse must be pretreated first to improve its digestibility and easy access for microbial attack (by removing core and noncore lignin fractions) (Pandey *et al*., 2000). Direct use of bagasse is not susceptible to exploit as substrate for cellulose (Javed *et al*., 2007).

Generally, the pretreatment process involves employing high temperature, pressure, acids or alkaline and organic solvents to disrupt the lignin seal and cellulose crystalline structure of lignocellulosic material. There are a few methods of pretreatments such as dilute acid pretreatments, alkali pretreatment, steam pretreatments, gamma radiation and enzymatic pretreatment.

Most of pretreatment methods have their own drawbacks in large scale application. For example, the dilute acid process generates toxic byproducts, such as furfural and aldehydes, which not only significantly reduces the sugar yield but also poisons enzymatic hydrolysis and biofuels fermentation. Steam explosion, operated at high temperature and pressure to achieve fibrillation, requires costly capital investment for equipments (Kuo and Lee, 2009).

In this study, the chemical pretreatment (alkaline pretreatment- e.g., treatment with alkali such as sodium hydroxide solution) is found to be effective and economical. Rodriguez-Vazguez *et al.* (1992) treated bagasse (pith) with a solution of sodium hydroxide in such a low volume that no free liquid was present. They referred it as dry pretreatment and compared it with wet pretreatment. Maximum digestibility with dry and wet pretreated bagasse was 75% and 71% respectively.

Schimper *et al*., (2009) also applied alkali pretreatment in the study on fabrics. Fabrics were pre-treated in one step which consists of immersing in alkali solutions for 1 or 2 min at a liquor ratio of 1:3 (w/v). No tension was applied to the fabrics. A range of concentrations (0 - 4.9 mol/L) was used in the impregnation solution. After squeezing in a padder, samples were rinsed two times with deionized water and then neutralized in a solution of 20 mmol/L acetic acid for five minutes. The alkali uptake was found to be between 100 and 250%, depending on alkali concentration. After neutralization the fabrics were exposed to enzyme for enzymatic hydrolysis.

2.3 Enzymatic Hydrolysis

Enzymatic hydrolysis is a catalytic decomposition of a chemical compound by reaction with water, such as the conversion of cellulosic material into fermentable sugars by the addition of specific enzymes. In this study, the cellulosic material used is sugarcane bagasse. It will be hydrolyzed into fermentable sugar which is glucose by addition of cellulase as the specific enzyme. Figure 2.3 shows the general pathway of enzymatic hydrolysis.

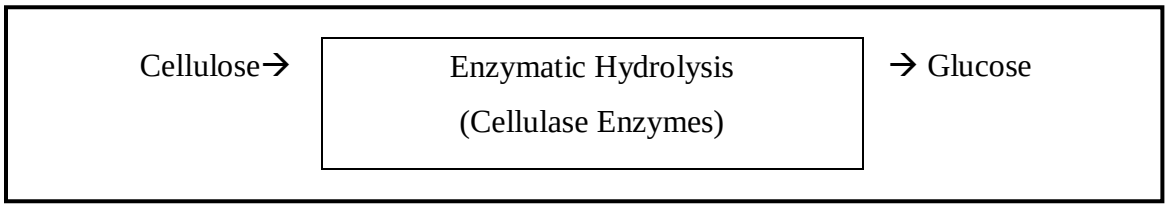

Figure 2.3: General pathway of enzymatic hydrolysis

This method is chosen as its utility cost is the lowest if compared to acid and alkaline hydrolysis. Sun and Cheng (2002) reported the enzymatic hydrolysis is usually conducted at mild conditions (pH 4.8 and temperature 45-50°C) and does not have corrosion problem.

Enzymatic hydrolysis of cellulose has become an important research area due to the potential use of cellulosic biomass as feedstock for fermentation into ethanol. The enzymatic breakdown of cellulose to fermentable sugars is done by enzymatic hydrolysis of the glucosidic bonds. The reaction is thus a two-substrate reaction involving both cellulose and water. While there has been considerable interest in the cellulose–enzyme interactions as well as on the cellulose composition, limited attention has been paid to the role of water in the process. Felby *et al*., (2008) investigated the cellulose-water interactions during enzymatic hydrolysis. During the initial enzymatic hydrolysis of cellulose, the action of the enzyme system is a breakdown and loosening of the cellulose introducing more water into the structure and providing better access for the enzymes.

Cellulases enzymes refer to enzymatic hydrolytic system consist of three different enzymes and act in specific way. First, an endoglucanase attacks one of the cellulose chains within the crystal structure, breaking the strand via hydrolysis and thereby exposing two new chain ends. During hydrolysis process, a molecule of water is consumed and one of the chain ends chemistry becomes “reducing” and the other “non-reducing”. Then an exoglucanase attaches to a loose chain end, physically pulls the cellulose chain away from the crystal structure and then proceeds to work its way down the chain, breaking off cellobiose (a dimeric sugar comprised of two glucose molecules) as it goes. Actually, there are two types of exoglucanase – a cellobiohydrolase I (CBH I) attaches to the “reducing” end and a cellobiohydrolase II (CBH II) attaches to the “non-reducing” end. Finally, a betaglucosidase splits the cellobiose molecule into two

separate glucose molecules, making them available for processing into chemicals or fuels (Lenting and Warmoeskerken, 2001).

The process in enzymatic hydrolysis had been reported in literature review over the years. Enzymatic hydrolysis on corn stalks was reported by Dale *et al*., (1995). The hydrolysis of the corn stalks with the cellulase was measured by taking 0.1 g of cellulose in the corn stalks sample added to 9.9 ml water. Then, 66.7 μL of cellulase enzyme was added to the solution, and the sample was allowed to digest at 50°C. Final glucose concentrations of 4.8 g/L glucose, 3.2 g/L cellobiose, and 2.6 g/L xylose were noted.

The enzymatic hydrolysis on waste paper office was done using three commercial cellulases; Acremonium cellulase, Meicelase and Cellulosin T2. The glucose percentage was measured more than 90% from various waste papers. The research was reported by Park *et al*. (2002). Najafpour and Shan (2002) investigated the enzymatic hydrolysis of molasses. The study was to increase the amount of fermentable sugar for ethanol production. The fermentable sugar content of molasses by enzymatic hydrolysis was increased from 194 to 611 g/L. The obtained sugar enriched molasses represents a better quality of feed stock for fermentation industries such as ethanol production.

2.4 Production of Glucose

A number of studies in production of glucose have been developed over the years. The productions of glucose involved enzymatic hydrolysis or by chemical hydrolysis have been reported in the journals. Chemical hydrolysis, usually acid hydrolysis, is one of the viable methods currently being developed as a promising means of producing sugar from cellulose. The hydrolysis of cellulose in mineral acids is strongly affected by the acid concentration and temperature (Sun *et al*., 2009).

Katz and Reese (1968) reported that the enzymatic hydrolysis of commercial cellulose (Solka Floc) can give concentrations of glucose (30%) comparable to those obtained in the enzymatic hydrolysis of starch. The glucose production was under optimal condition for 70 hours and enzymes used were cellulase from *Trichoderma viride* plus β-glucosidase. The incubation was at pH 4.5 and temperature 40°C. It approves that enzymatic hydrolysis on cellulose can produce glucose.

Mosier *et al*., (2002) studied about the characterization of acid catalytic domains for cellulose hydrolysis and glucose degradation. The hydrolysis of cellulose in corn fiber was tested using maleic and sulfuric acids at 50 mM concentrations. The corn fiber was first pretreated by pressure cooking it in water, followed by addition of the appropriate amount of acid. After hydrolysis at 160°C for 30 minutes, chromatograms of the liquid recovered from hydrolysis show that significant glucose has been generated. The result showed that the highest yield of glucose was obtained by using maleic acid which is 26 g and by using sulfuric acid, the glucose was only 22.3 g. Maleic acid possesed superior selectivity for the production of fermentable sugars from cellulose more than than sulfuric acid.

2.5 The Application of Glucose in Industry

Glucose benefits much for industry, medical field and researches. Numbers of applications have been reported up to now. Shen and Xia (2006) produced lactic acid from industrial waste corn cob. The corn cob was hydrolyzed by cellulase and cellobiase. The cellulosic hydrolysate contained 52.4 g L^{-1} of glucose and was used as carbon source for lactic acid fermentation by cells of *Lactobacillus delbrueckii* ZU-S2 immobilized in calcium alginate gel beads. The final concentration of lactic acid and the yield of lactic acid from glucose were 48.7 g L^{-1} and 95.2%, respectively, which were comparative to the results of pure glucose fermentation.

From medical field, the increases in glucose production that occur during infusion of epinephrine may result in part from a direct effect of epinephrine on the liver as well as from effects of epinephrine on insulin secretion, glucagon secretion, and gluconeogenic precursor availability. The decreases in glucose clearance observed during infusion of epinephrine may be due in part to direct inhibition of tissue glucose uptake by epinephrine and to inhibition of tissue glucose uptake secondary to effects of epinephrine on plasma insulin and free fatty acid concentrations. Furthermore, when assessment of these adrenergic mechanisms is attempted in vivo by infusing either an alpha or beta adrenergic antagonist along with epinephrine, alteration in circulating hormone and substrate concentrations (e.g., insulin and glucagon, or insulin and free fatty acids) occur that may have opposing effects on glucose production or clearance (Rizza *et al*., 1980).

The concept of mass balance was used to analyze the metabolic pathways of citrate production by *Candida lipolvtica* from glucose (Alba and Matsuoka, 1979). Specific rates of glucose consumption, citrate and isocitrate productions, carbon dioxide evolution and cellular syntheses of protein and carbohydrate were observed in an NH_4^+

limited chemostat culture. These data permitted one to assess the carbon flux *in vivo* by solving simultaneous carbon balance equations with respect to intermediary metabolite pools in the steady state.

2.6 Factor Affecting the Production of Reducing Sugar

A reducing sugar is any sugar that has an aldehyde or a ketone group. This includes glucose, fructose, glyceraldehyde and galactose. The maximum production of reducing sugar is affected by various conditions such as effect of pH, revolution per minute (rpm), time and so on. In this experiment, the effect of temperature, effect of substrate dose and effect of enzyme dose in glucose production from sugarcane bagasse were investigated. Some literature reviews reported on these conditions on production of reducing sugar.

2.6.1 Effect of Temperature on Reducing Sugar Production

The effects of temperature in the range 40°C to 60°C were investigated on the enzymatic hydrolysis of steam-pretreated willow to obtain the optimal hydrolysis conditions for production of glucose (Eklund *et al*., 1990). The temperature affects both the initial hydrolysis rate and the final glucose yield. The highest glucose yield was obtained at 40°C. The yield will decrease with increasing temperature, due to the increased of enzyme deactivation at higher temperatures, while the initial reaction rate increased with increasing temperatures.